Diagnostic criteria in invasive pituitary adenomas

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Abstract: Pituitary adenomas are benign pituitary primary tumors, the most frequent type of tumor in the pituitary fossa. An important part, around 1/3 of the pituitary adenomas manifests an aggressive behavior, growing faster and invading into parasellar areas (cavernous sinus, neural tissues and bones). *Objectives*: the first aim of this paper is to review the last findings about invasiveness diagnostic criteria, imagistic and biomarkers, which can be used in the classification of pituitary tumors and also to predict the probability of invasiveness, tumor recurrence and suspicion of malignancy. The second aim is to highlight the morphological and clinic types of invasive pituitary adenomas. *Materials and methods*: we performed a systematic review and analysis of the published articles, searching PubMed between January 1985 and December 2015. There were selected articles published in English, reviews and abstracts. During the advanced search type in PubMed, combinations of the following keywords were used: "pituitary adenoma", "invasive", "aggressive", "biomarkers", "classification", "histological subtypes", "'immunohistochemical markers". *Results*: 215 articles were selected, regarding diagnostic, prognostic and therapeutic aspects. There were some histological subtypes of pituitary adenomas known as having an aggressive clinical behavior. Several biomarkers were identified as being associated with the invasive feature: proliferation markers (Ki-67 index, number of mitoses, p53 & p27 expression, microvascularization density, telomerase, topoisomerase 2 Alpha), matrix metalloproteinases, protein kinase C, cyclooxygenase-2, E-cadherin, transcription Factors, genetic alterations (PTTG gene, Galectin-3 protein/ LGALS3 gene), apoptosis markers. Based on their invasion and proliferation characteristics, pituitary tumors are proposed to be classified into five grades (1a, 1b, 2a, 2b, 3), the grade 2b tumor with high risk of recurrence being considered as tumor suspected of malignancy. *Conclusions*: Using a set of specific biological markers for invasive process, there is hope to establish an early diagnosis and prevention of invasive pituitary adenomas. Due to the fact that aggressive pituitary

tumors are generally difficult to manage, unresponsive to therapy, quickly recurrent and associated with poor prognosis, the early diagnosis and the search for new therapeutic approaches is becoming mandatory. Instead of using "invasive" or "aggressive" adenoma, the term "tumor suspected of malignancy" would be used for more accuracy.

Key words: pituitary adenoma, invasive, aggressive, biomarkers, classification, histological subtypes, immunohistochemical markers

Introduction

The pituitary primary tumors, originating from adenohypophyseal cells, can be benign (adenomas) and malignant (carcinomas).

The pituitary adenomas' incidence is about 10–15% of all brain tumors, being on the third place, after gliomas and meningiomas. The pituitary carcinomas represent only 0.1% of all pituitary tumors (1).

Invasion represents the phenomenon in which the cells from a malignant neoplasm extend to the adjacent healthy tissues, infiltrating and destructing them. For epithelial neoplasms, invasion signifies infiltration beneath the epithelial basement membrane. Although pituitary adenomas are non-metastasizing tumors, invasive local growth occurs in 35% of cases (2).

Atypical adenomas are small subset of pituitary adenomas, tumors with a more aggressive behavior, which have potential to grow faster than typical adenoma and have also potential of invasion into vascular (cavernous sinus), neural tissues and bones (1).

Most of the neurosurgeons agree that a key feature in defining an "aggressive" pituitary tumor is the rapidity of growth, which often may cause an early recurrence. A tumor that re-grow in 6 months is considered "aggressive", compared with a recurrence in 10 years after a complete resection, which might be considered as a benign behavior (2).

Materials and methods

An extensive medical literature research was performed in Pubmed, years of publication from January 1985 – December 2015. The analyzed articles were published in English language, in abstract or in extenso and 20 articles were excluded. There were selected articles focusing on classification of pituitary tumors, biomarkers of invasiveness, diagnostic criteria, morphologic types of invasive pituitary tumors, imagistic criteria, on tumor recurrence and suspicion of malignancy.

For the research, there were used combinations of 2 or 3 of the biomarkers identified as being associated with the invasive feature: proliferation markers (Ki-67 index, number of mitoses, p53 & p27 expression, microvascularization density, telomerase, topoisomerase 2 Alpha), matrix metalloproteinases, protein kinase C, cyclooxygenase-2, E-cadherin, transcription Factors, genetic alterations (PTTG gene, Galectin-3 protein/ LGALS3 gene), apoptosis markers.

Results

From clinical point of view, in the studied articles was found that the pituitary tumors are

classified into functioning – acromegaly syndrome with growth hormone (GH) secretion, or amenorrhea-galactorrhea with prolactin (PRL) secretion, or Cushing's disease with adrenocorticotropic hormone (ACTH) secretion) and nonfunctioning tumors – secreting mainly follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The functional tumors represent 75% of pituitary adenomas, but the non‑secreting tumors are usually larger (3).

Beside the increased secretion of some hormones, the pituitary tumors are usually causing a subsequent "mass effect" on surrounding structures (optic chiasm and/or the cavernous or sphenoid sinuses), with symptoms and signs such as: headaches; epistaxis (due to downward extension through the floor of sella) (4); visual field impairment (typically bi‑temporal field loss or diplopia) or even proptosis (due to a mass extended to orbit) or other neurological deficits (cranial nerve palsies, due to invasion into cavernous sinus) (4). Headaches or vision loss with sudden onset can be due to hemorrhage or necrosis of tumor (5).

In the studied articles we identified several hormone-secreting and morphological subtypes of pituitary adenomas, which tend to have a more aggressive clinical behavior. These include: 1) aggressive prolactin-secreting pituitary tumors (sparsely granulated somatotroph and acidophil stem cell adenomas); 2) aggressive corticotroph pituitary tumors (silent corticotroph adenomas, thyrotroph adenomas, Crooke's cell adenomas); 3) aggressive growth-hormone secreting pituitary tumors(more common are densely and sparsely granulated somatotroph adenomas); 4) aggressive gonadotroph and thyrotroph pituitary tumors (tumors immunohistochemical positives for the FSH and/or LH gonadotrophins or the oncocytomas); and 5) aggressive plurihormonal tumors (silent subtype 3 adenomas).

Tumor invasion was defined based on one or more of the following parameters: preoperative imaging (MRI or CT), intraoperative findings, and histology (6). By size, pituitary adenomas are classified by size into microadenoma (b<10 mm) and macroadenomas (≥10 mm).

It was shown that the invasion of the cavernous sinus space occurs in 6 to 10% of all pituitary adenomas. It is only considered an unequivocal invasion of the cavernous sinus, when the percentage of encasement of the internal carotid artery by the tumor is 67% or greater, or for grades 3 or 4 of Knosp's classification (7). In case of such large invasive macroadenoma, the total surgical resection is unobtainable.

The proliferation markers for aggressive pituitary tumorsidentified were: Ki-67 index, number of mitoses, p53 & p27 expression, microvascularization density, telomerase, topoisomerase 2 Alpha. The main diagnostic criteria for atypical pituitary adenoma, included in all the studied articles, included: pleomorphism, elevated mitotic index, increased nuclear reaction for the p53 protein, and a Ki-67 proliferative index >3% (8).

Increasing levels of Ki-67 correlate with rate of tumor growth, invasion, responsiveness to pharmacological treatment, tumoral

remission and recurrence (8). A threshold of 3% Ki-67 index can distinguish between invasive and noninvasive adenomas, with 97% specificity and 73% sensitivity and has indeed a prognostic value (9). The mean values of Ki-67, reported by Thapar et al. (1996) were 1.37% in noninvasive pituitary adenomas, 4.66% in invasive adenomas and 11.91% in carcinomas (9).

A number of mitoses higher than 2/10 HPFs (10 HPF: high power field=2 mm2, at least 40 fields, at 40× magnification, evaluated in areas of highest mitotic density) indicate the proliferative characteristic of a tumor (10).

Several studies showed an increased p53 expression in 'aggressive-invasive' pituitary tumors: significantly higher (p=0.0001) or of 15% in invasive pituitary adenomas and of 100% in pituitary carcinoma metastases (11, 12).

p27 KIP1 expression was found lower in recurrent adenomas compared with non-recurrent ones, especially in corticotroph adenomas, and in carcinomas compared with invasive adenomas (13).

In invasive pituitary tumors Vidal et al. described a higher microvascular density, but without statistical significance (14). Also, Salehi et al. obtained inconsistent association between the expression of vascular endothelial growth factor (VEGF) and the tumor invasiveness and proliferation (15).

Harada et al. (16) and Yoshino et al. (17) detected a telomerase expression in 13% of large, invasive adenomas, therefore telomerase detection may be also useful for identifying aggressive adenomas.

Also, in pituitary adenomas, there were reported low values of topoisomerase II alpha, results similar to those of Ki-67, however further studies are requested to establish if this enzyme is a valid marker of tumor aggressiveness (18).

Other characteristics modifications in aggressive pituitary tumors identified in the studied articles were: matrix metalloproteinases, protein kinase C (PKC), cyclooxygenase-2, E-cadherin, genetic alterations (PTTG gene, Galectin-3 protein/ LGALS3 gene), apoptosis markers.

In aggressive/invasive pituitary tumors, it was demonstrated that MMP9 expression level and activity is higher than in noninvasive pituitary tumors (19).

In invasive pituitary adenomas was shown by a point mutation of PKC-α and a higher total PKC activity and expression (20) and in some cases of prolactinomas that responded favorably to dopamine agonists therapy was observed a reduced PKC activity.

Cyclooxygenase-2 (COX-2) presents an increased expression evident particularly in pituitary carcinomas, compared with adenomas and normal pituitary (21),

In a study of van Roy and Berx it was observed that in pituitary aggressive/invasive adenoma, cells E-cadherin and β-catenin expression was downregulated and a decreased E-cadherin expression can lead to development of metastases (22).

Also, they were demonstrated accumulations of new chromosomal alterations which may transform an "aggressive" pituitary tumor into a pituitary carcinoma, as in the case of PRL aggressive tumors (grade 2b), that became malignant and

developed metastasis during follow-up (the 11p region was usually deleted, the 11q arm was loss and in the 1q arm was a gain) (23).

Zhang et al. noted significantly higher levels of pituitary tumor transforming gene (PTTG) expression in hormone-secreting invasive tumors compared with non-invasive ones (24).

In both PRL and ACTH functioning pituitary tumors (adenomas and carcinomas) was shown that LGALS3 has a higher expression, with the highest level in ACTH carcinomas (25).

Higher apoptotic activity was reported in aggressive, drug-resistant adenomas by Kontogeorgos et al. (26) and in pituitary carcinomas compared with adenomas, Kulig et al. (27) reported a four-fold increase in apoptotic activity.

Discussions

Imagistic diagnostic criteria for aggressive pituitary tumors

Recent anatomical and ultrastructural studies have shown that the medial wall of the cavernous sinus is composed of dura. Dural invasion is not considered a feature of invasion because previous studies demonstrated that it is not related to the recurrence rate. Suprasellar growth is considered an extension, rather than an invasion. According to Hardy's neuroradiological classification, tumors with a suprasellar expansion that were frequently lined by non-tumoral pituitary, are not considered as invasive.

Any tumor growth into the cavernous sinus would therefore represent a sign of invasiveness without histological proof, such as the invasion into the sphenoid sinus confirmed by the infiltrated respiratory mucosae on histology.

New methods of immunohistochemistry, by staining with KI-67, a significantly higher proliferation rate were demonstrated in invasive adenomas; this was not only true in pituitary adenomas with histologically proven invasion of the dura, but also in pituitary adenomas macroscopically invading the cavernous sinus space (28).

MRI importance

MRI offers detailed information on the anatomical relationship of the tumor with the surrounding structures; therefore, it is considered as the method of choice in the diagnosis of pituitary adenomas and it also become the gold standard for diagnosis and follow-up of pituitary adenomas. The application of gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) enhances precisely the cavernous venous plexus, which allow the precise distinction between the medial, superior, inferior, and lateral compartments (29).

Although it is common to wait 3–4 months after surgery to obtain the first MRI, even immediate postoperative imaging is useful to provide information regarding the presence of residual tumor, in order to start early specific therapeutic strategies.

But despite the MRI improvements, there are authors who consider that the invasion into the cavernous sinus space cannot be proved only by MRI and that the invasion of the cavernous sinus space remains a surgical diagnosis, because only the surgeon can distinguish between compression of the

venous spaces and real infiltration of the medial wall of the cavernous sinus space (30).

Proliferation markers for aggressive pituitary tumors

There are evidences at the molecular level which indicate that pituitary tumors accumulate abnormalities over time, fact that contribute to their progression from "benign" adenoma to aggressive recurrent pituitary tumors and even to a pituitary carcinoma (15).

Proliferation is defined as the presence of at least two of the three markers: mitoses or Ki-67 index which exceeded the defined thresholds, or positive p53 detection.

Ki-67 index

The protein Ki-67 is a marker of cell proliferation present in the nuclei of cells in G1, S, and G2 cell division cycle phases and in mitosis. The Ki-67 expression is detected by the monoclonal antibody MIB-1 and is quantified as Ki-67 proliferation index being a percentage of immunopositive nuclei (31).

The Ki-67 index >1 or C3% according to the Bouin-Hollande or formalin fixative and the number of mitoses n>2/10 HPFs had been defined previously in pituitary and/or endocrine tumors (10). For pituitary adenoma, Ki-67 is a routinely examined marker of cell proliferation.

In clinical practice, Ki-67 index is daily used to select the patients who need a strict follow-up, because a very high proliferation index suggests the presence of a carcinoma in situ or premetastatic carcinoma, with potential for a rapidly progressive and fatal course. The above mentioned data have led to a proposal that pituitary tumors exhibiting Ki-67 index greater than 10% should routinely be classified as atypical independent of other criteria (21).

Even if Ki-67 index >3% is considered a good proliferation marker, its values exhibit a certain variability. They vary depending of immunohistochemical (IHC) type and in functional pituitary tumors are higher than in nonfunctioning ones. Also, there are authors who found no correlation between Ki-67 index and pituitary tumor invasiveness (21). Therefore, it is recommended the use of other makers for more accurate prediction of the tumor behavior.

Number of mitoses

Beside Ki-67 index, also the presence and the number of mitoses can represent important predictive factors of proliferation and rapid growth in endocrine tumors.

p53 expression

p53 is a tumor suppressor gene that plays an important role in cell proliferation, apoptosis and genomic stability. There are also reports about a non-conclusive correlation between p53 increased expression and aggressive adenoma behavior (31), but it is possible to exist differences in p53 evaluation between laboratories and pathologists. Therefore, only the p53 positivity or negativity is considered, because, actually, there is no validated prognostic cut-off for it (11), but a p53 positive immune reaction has been found in all pituitary carcinomas (9,11) and, also, p53 is one of the criteria to classify "atypical adenomas" (6).

p27 expression

p27 KIP1 is a cyclin-dependent kinase inhibitor, involved in regulation of cell-cycle

progression.Because p27 KIP1 expression was inversely correlated with the Ki-67's one, it was suggesting that p27 KIP1 is an additional predictive marker of pituitary tumor behavior (32).

Telomerase

Telomerase is a reverse transcriptase enzyme carrying its own RNA molecule, which elongates telomeres and contributes to the preservation of the senescence-crisis-apoptosis cycle. Because this represent one of the fundamental defense mechanisms against cancer development, this can be a valuable exploration in aggressive pituitary tumors.

Topoisomerase 2 Alpha

Topoisomerase II alpha is a key enzyme involved in DNA replication and cell-cycle progression, not found in resting cells; its expression correlates with cell proliferation (18), fact which can also be valuable in exploring aggressive tumor comportment.

Other characteristics modifications in aggressive pituitary tumors

Angiogenesis

The "angiogenic switch", the imbalance between stimulating and inhibiting angiogenetic factors, becoming predominant promoting factors, represents an important phenomenon in tumorigenesis: the formation of new blood vessels sustains the tumor growth.

It was observed that in pituitary adenomas, they are less vascularized than the normal pituitary tissue; this can explain the slow growth and lack of metastasis, and lead to the development of the hypothesis that pituitary adenoma evolve through a non-angiogenic pathway. In carcinomas it was observed a higher vascularization than in the adenomas, finding which supports the idea that the development of metastasis is correlated with the neo-angiogenesis (33).

But nevertheless, VEGF is an important angiogenic factor up-regulated by PTTG and future studies could confirm its link with invasive pituitary adenomas (15).

Other enzymes

Matrix metalloproteinases

The matrix metalloproteinases (MMP) are a family of proteolytic enzymes cleaving the extracellular matrix molecules. MMPs play a role in invasiveness of many neoplasms and the MMP study underlines the importance of the pituitary tumor environment.

The expression of MMP9 may be correlated with the activation of protein kinase C (PKC), which is also known to indicate invasion and aggressive behavior of pituitary tumors (34).

Protein kinase C

PKC is a ubiquitous family ofisoform enzymes, playing important roles in controlling the function of other proteins and in signal transduction cascades. Therefore, PKC are associated with a variety of cellular responses, including cell growth and invasion (19).

PKC activation has been shown to increase MMP-9 expression in some tumor cells. Because PKC activates MMP-9 in a highly cell-type-specific manner, the differential expression of PKC isozymes may involve various signal transduction pathways; therefore, it is important to identify which

PKC isozyme is regulating the MMP-9 expression of in pituitary adenomas (19).

Cyclooxygenase-2

COX-2 is a key enzyme of prostaglandin synthesis, involved in inflammatory responses. There were studies who reported that COX-2 presents an increased expression also in the gonadotrophic tumors, compared with other pituitary neoplasms (35). The observations sustain the COX-2 implication in tumor invasiveness, angiogenesis and progression; indeed, it was observed that COX-2 expression shows a strong correlation with microvessel density (21).

E-Cadherin

E-cadherin is a calcium-dependent cell adhesion protein, playing an important role in epithelial cell behavior and tissue development and having a strong anti-invasive and anti-metastatic role. In normal pituitary cells E-cadherin is strongly expressed (22).

Transcription Factors/regulators

For immunonegative or silent tumors, the transcription factors involved in pituitary cell differentiation may be used to confirm the diagnosis. Pit-1 is expressed in GH, PRL, and thyroid stimulating hormone (TSH) tumors; T-pit in ACTH tumors with and without Cushing's disease and SF-1 in FSH and LH tumors (36).

Rarely, some pituitary tumors differentiate functionally and produce hormones belonging to different cell lineages (ACTH-omas with GH production, or GH-omas with ACTH production); this abnormal differentiation could be caused by an aberrant expression of transcription factors (37).

Ikaros is a transcriptional regulator and an important factor implicated in chromatin remodeling that can influence hypothalamic-pituitary cell development, differentiation and proliferation (38).

Genetic alterations

Despite the pituitary tumors are in majority sporadic, they characterized some genetic forms of pituitary adenoma in familial cases.

PTTG gene

The pituitary tumor transforming gene (PTTG) is another less well studied marker, found in 90% of pituitary tumors. 2.9% or more of PTTG expression can be also considered an indication of a more aggressive behavior of pituitary adenomas.

Comparing PTTG expression in 54 pituitary tumors, Zhang et al. found no correlation with radiological tumor stage in clinically non-functioning adenomas, even they founded significantly higher levels in invasive tumors (24).

H-ras gene mutation

Several proto-oncogenic events, as the p53 and p27kipl expression, the function of telomerase, and the role of H-ras gene mutation, can represent central events in the pathogenesis and spread of pituitary carcinomas (15, 16, 17).

Galectin-3/ LGALS3 gene

Galectin-3 is a protein encoded by the LGALS3 gene, expressed in pituitary gland by both folliculostellate cells and normal prolactin (PRL) and adrenocorticotropin (ACTH)-producing cells, but not by most other cell types. LGALS3 is a useful

immunohistochemical marker for differentiating silent from functioning ACTH-subtype adenomas.

LGALS3 represents a reliable marker for predicting the aggressive tumor behavior (assessing a high risk of progression or recurrence) (12). PRL and ACTH functioning pituitary tumors (adenomas and carcinomas) are aggressive subtypes of tumors, in which the invasive growth with suprasellar extension, a high Ki-67 index, and LGALS3 expression levels are the most important pathologic features, therefore a target therapy against Galectin-3 protein may be useful.

Apoptosis

Apoptosis, the programmed cell death, is a sequence of events characterized by cellular shrinkage and nuclear demarcation, which ends with the elimination of damaged cells. In neoplasms, there is a misbalance between mitotic and apoptotic activity, apoptosis is generally suppressed and there is an increased tumor growth (15).

Apoptosis may be a useful prognostic marker; the expression of anti-apoptotic factor BCL2 and proapoptotic factor BAX also correlates with apoptotic indices. A lower expression of BCL2 was also reported in pituitary carcinomas compared with adenomas and the non-tumoral pituitary gland (27).

The new classification criteria for atypical adenomas

Until the 1980's, the classification of pituitary tumors included three types, based on tinctoriality correlated with the clinical disease: acidophilic with acromegaly, basophilic with Cushing's disease, and chromophobic adenomas. The advancements in electron microscopy (EM) and immunohistochemistry (IHC) changed the classification of pituitary tumors into five immunocytochemical types and a dozen ultrastructural subtypes, based on their organelles appearance (granulations and mitochondria) and their hormonal secretion (39).

The new 2004 WHO classification for endocrine tumors define 3 types of pituitary tumors: benign adenoma, atypical adenoma, and carcinoma (1,8).

Wolfsberger and Knosp criticized the WHO 2004 classification; they stated that "the definition of invasiveness is needed and should be included in this classification" and underline the need to also include proliferation, evaluated by markers of the cell cycle with well-defined thresholds (40). Considering invasion potential for atypical adenomas at a threshold of Ki-67 index >3% and p53 >5%, Saeger et al. found only 2.7% of among 241 tumors from the German registry (1).

Actually, it is recognized that there is no reliable distinction between carcinoma and adenoma based on distinct standard histological criteria or electron microscopic features, distinct that would permit a reliable early prediction of future aggressive behavior of a pituitary adenoma, even if invasive.

Based on above mentioned characteristics of atypical adenomas, recently, a new clinicopathological classification has been proposed, which takes into account tumor size, immunocytochemical type (hormonal immunoexpression profile), tumoral invasion

(invasion to the cavernous and sphenoid sinuses, evaluated by magnetic resonance imaging (MRI) or histology), and tumor cell proliferation markers (Ki-67 and p53) (41).

Therefore, based on their invasion and proliferation characteristics, the tumors were classified into five grades: grade 1a: non-invasive tumor, grade 1b: non-invasive and proliferative tumor, grade 2a: invasive tumor, grade 2b: invasive and proliferative tumor, grade 3: metastatic tumor).

Trouillas proposed to consider the grade 2b tumors with high risk of recurrence as tumors suspected of malignancy (41).

The aggressive subtypes of pituitary adenomas

Aggressive prolactin-secreting pituitary tumors

Prolactinomas represent about 40% of all pituitary adenomas. Two histological subtypes are clinically relevant (42):

1 – the sparsely granulated lactotroph adenoma - is the most common form, more aggressive in men than in women, which can reach considerable size, high PRL serum levels and invasive features at MRI. In many cases, this type responds dramatically to dopamine agonist therapy;

2 – the densely granulated lactotroph adenoma, with diffuse cytoplasmic positivity to PRL - it is very rare;

3 – the acidophil stem cell adenomas - presents weakly acidophilic cells producing PRL and GH, oncocytic changes, and giant mitochondria. It often has an aggressive clinical behavior, with hyperprolactinemia and/or acromegaly (1).

Clinically, some mass effect features are observed, such as visual field restriction or dull headache at presentation and symptoms of amennorrhea and galactorrhea in females and erectile dysfunction in males.

The aggressive prolactinomas present serum PRL levels which can vary widely (6–21,560 ng/ml in one series). Imagistic studies often put into evidence a pituitary macroadenoma with invasion of one/both cavernous sinuses, which extent and often encase the internal carotid arteries (43,44).

Aggressive corticotroph pituitary tumors

10–15% of all pituitary adenomas are ACTH-secreting adenomas. Two morphologic variants have been identified at electron microscopy level: subtype 1 - densely granulated basophilic tumors, similar to functional ACTH secretory tumors and subtype 2 - different by electron microscopy, chromophobic, sparsely granulated and their secretory granules are smaller and irregular in shape and lack cytoplasmic intermediate filaments (45).The densely granulated corticotroph subtype is the most common (42,45), usually present in patients with Cushing's disease and Nelson's syndrome.

Crooke cell tumors is a rare form of ACTH producing adenoma, tumor cells presenting a variable but often characteristic Crooke's intracytoplasmic accumulation of cytokeratin. Clinicopathologically, these tumors represent a distinct entity from typical endocrinologically active corticotroph adenomas; they may produce ACTH-causing Cushing's disease or may be endocrinologically silent. Pituitary tumors composed of Crooke cells exhibit aggressive clinical behavior, with high recurrence rate,

and invasiveness (46), and nearly always these recurrent invasive macroadenomas may progress to corticotroph carcinomas (46). The Crooke's cell adenoma must not be confused with keratin deposition in the normal corticotrophs (Crookes hyaline change), first described in 1935 by endocrinologist AC Crooke.

Clinically, patients with aggressive pituitary corticotroph tumors present Cushing's disease, with ACTH dependent hypercortisolism, including central obesity, rounded facies, abdominal and proximal limb striae, hypertension, altered menses, osteoporosis and delayed wound healing (40).

A second clinical onset is observed in Nelson's syndrome, the rapid enlargement (likely to occur within the first 3 years) of a pre-existing ACTH-secreting pituitary adenoma that occurs after bilateral adrenalectomy to control hypercortisolismin patients with Cushing's disease (47).

Thirdly, they are recurrent but "silent" corticotroph tumors, which present variable immunopositivity for ACTH. Clinically, are nonfunctional adenomas, which do not secrete excess ACTH, but have a more aggressive course than most functional ACTH-secreting tumors (48) and recur more frequently than tumors with hypercortisolism (48).

Aggressive growth-hormone secreting pituitary tumors

GH-secreting adenomas represent 10–15% of all pituitary adenomas and are classified into five histological subtypes; the more common are densely and sparsely granulated somatotroph adenomas (42).

Usually, these adenomas are small or medium in size, and GH serum levels are moderate increased; they respond to treatment with long-acting somatostatin analogs. Quantification of somatostatin receptors 2 and 5 (SSTR2, SSTR5) can be useful to predict the response to medical treatment (49): all tumors (GH, TSH, ACTH, and FSH-LH secreting) with a low SSTR2 expression are at a higher risk of resistance to octreotide / lanreotide and those with high SSTR5R expression are more likely to respond to pasireotide treatment. This detection is always negative in PRL tumors (49).

The sparsely granulated somatotroph adenomas are larger and more invasive (proved by MRI); this lead to an incomplete resection and in some cases, they are resistant to long-acting somatostatin analog therapy (50).

Aggressive gonadotroph and thyrotroph pituitary tumors

Almost all pituitary tumors (up to 97 % in some studies) are immunohistochemical positive for the FSH and/or LH gonadotrophins, even 30–35 % of them are clinically nonfunctioning (do not cause a clinical endocrine syndrome). Often those tumors are large invasive macroadenomas, with pressure on the optic chiasm, varying degrees of hypopituitarism; and FSH and LH serum levels which may be elevated or not (51). The clinically nonfunctioning tumors are rarely encountered as aggressive tumors, but can behave aggressively and they were reported cases of clinically nonfunctioning pituitary carcinoma.

The oncocytomas are FSH-LH adenomas with high concentration of mitochondria.

The null cell adenomas are tumors immunonegatives for adenohypohysialhormones, they represent only 1% from all pituitary tumors.

TSH-producing adenomas are less than 2% of all pituitary adenomas (1, 42, 45).

Aggressive plurihormonal tumors

The silent subtype 3 adenomas are plurihormonal tumors, usually immunohistochemical positive for GH, PRL and TSH, with nuclear inclusions called spheridia (electron microscopy revealed). Clinically, they are usually silent, but may be associated with hyperprolactinemia, acromegaly or hyperthyroidism. These tumors can also have an aggressive behavior (52).

Conclusions

For pituitary gland, it would be more correctly from oncological point of view (adenoma means benign) to use instead of "atypical" or "aggressive" adenoma, the term "tumor suspected of malignancy" (tumor grade 2b).

There is not a single specific biomarker able to confidently predict the aggressive/invasive behavior for pituitary tumors.

The correct histological diagnosis of pituitary adenomas may identify adenomas' subtypes known as having an aggressive clinical behavior: sparsely granulated somatotroph, acidophil stem cell, silentcorticotroph, with Crooke's cell, thyrotroph or silent subtype 3.

There is hope that establishing specific biological markers for invasive process, would became possible an early diagnosis and prevention of invasive pituitary adenomas.

Because aggressive pituitary tumors are difficult to manage, generally unresponsive to therapy, tend to recur quickly and are associated with poor prognosis, the early diagnosis and the search of new therapeutic approaches is becoming mandatory.

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